

CLEAN VERSION OF REWRITTEN OR ADDED CLAIMS  
PURSUANT TO 37 CFR § 1.21 (c)(1)(i)

Please cancel Claims 1-8, 10-12, 14-22, 24-26, 28, and 35-36.

Please amend the following Claims.

37. (Twice amended) A method of producing an allelic series of modifications in a gene of interest in a cell, comprising:

a) providing:

i) an *in vitro* culture comprising isolated mouse embryonic stem cells comprising a gene of interest;

ii) a chemical agent capable of producing at least one modification in said gene of interest;

b) treating said mouse embryonic stem cells with said chemical agent under conditions such that (i) it is at least 70% probable that at least one modification in substantially every gene in said mouse embryonic stem cells is produced, and ii) a mixture of embryonic stem cells comprising said gene of interest is produced, said mixture of embryonic stem cells comprising embryonic stem cells having a first modification in said gene of interest, and embryonic stem cells having a second modification in said gene of interest; and

c) isolating said embryonic stem cells having a first modification in said gene of interest and said embryonic stem cells having a second modification in said gene of interest, thereby producing an allelic series of modifications in said gene of interest in the isolated embryonic stem cells.

39. (Amended) The method of Claim 37, wherein said treating is under conditions such that it is at least 85% probable that at least one modification in substantially every gene in said mouse embryonic stem cells is produced.

40. (Amended) The method of Claim 37, wherein said treating is under conditions such that it is at least 95% probable that at least one modification in substantially every gene in said mouse embryonic stem cells is produced.

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46. (Twice amended) A method of producing an allelic series of modifications in a gene of interest in a cell, comprising:

- a) providing:
    - i) an *in vitro* culture comprising isolated mouse embryonic stem cells comprising a gene of interest;
    - ii) *N*-ethyl-*N*-nitrosourea;
  - b) treating said mouse embryonic stem cells with said *N*-ethyl-*N*-nitrosourea to produce treated mouse embryonic stem cells comprising a mixture of embryonic stem cells, said mixture comprising embryonic stem cells having a first modification in said gene of interest, and embryonic stem cells having a second modification in said gene of interest, wherein the treatment is under conditions such that the frequency of mutation in any one gene in said treated mouse embryonic stem cells is from 1/600 to 1/9000;
  - c) isolating said embryonic stem cells having a first modification in said gene of interest and said embryonic stem cells having a second modification in said gene of interest, thereby producing an allelic series of modifications in said gene of interest in the isolated embryonic stem cells; and
  - d) detecting at least one of said first and second modifications in said gene of interest using fluorescent chemical cleavage of mismatch.
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Please add the following claims.

51. (New) A method of producing an allelic series of modifications in a gene of interest in a cell, comprising:

a) providing:

- i) an *in vitro* culture comprising between 200 and 600 isolated mouse embryonic stem cells, each of said cells comprising a gene of interest;
- ii) a chemical agent capable of producing at least one modification in said gene of interest;

b) treating said mouse embryonic stem cells with said chemical agent under conditions such that (i) it is at least 70% probable that at least one modification in substantially every gene in said mouse embryonic stem cells is produced, and ii) a mixture of embryonic stem cells comprising said gene of interest is produced, said mixture of embryonic stem cells comprising embryonic stem cells having a first modification in said gene of interest, and embryonic stem cells having a second modification in said gene of interest[, wherein said treating is under conditions such that at least one modification in at least 70% of the genes in said mouse embryonic stem cells is produced]; and

c) isolating said embryonic stem cells having a first modification in said gene of interest and said embryonic stem cells having a second modification in said gene of interest, thereby producing an allelic series of modifications in said gene of interest in the isolated embryonic stem cells.

52. (New) The method of Claim 51, wherein said treating is under conditions such that it is at least 85% probable that at least one modification in substantially every gene in said mouse embryonic stem cells is produced.

53. (New) The method of Claim 51, wherein said treating is under conditions such that it is at least 95% probable that at least one modification in substantially every gene in said mouse embryonic stem cells is produced.

54. (New) The method of Claim 51, further comprising step d) placing at least one embryonic stem cell selected from the group consisting of said embryonic stem cells having a first modification in said gene of interest and said embryonic stem cells having a second modification in said gene of interest into an environment under conditions so as to generate a mouse comprising a modification selected from the group consisting of said first modification in said gene of interest and said second modification in said gene of interest.

55. (New) The method of Claim 51, wherein said gene of interest is associated with a disease.

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Cont  
56. (New) The method of Claim 55, wherein said gene of interest is selected from the group consisting of the p53 gene, BRCA1 gene, PKD1 gene, PKD2 gene, and PKD3 gene.

57. (New) The method of Claim 51, further comprising step d) detecting at least one of said first and second modification in said gene of interest using fluorescent chemical cleavage of mismatch.

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